A Section 508–conformant HTML version of this article is available at http://dx.doi.org/10.1289/ehp.1409535

Endocrine-Disrupting Chemicals and Oil and Natural Gas Operations: Potential Environmental Contamination and Recommendations to Assess Complex Environmental Mixtures

Christopher D. Kassotis,[1] Donald E. Tillitt,[2] Chung-Ho Lin,[3] Jane A. McElroy,[4] and Susan C. Nagel[5]

[1]Nicholas School of the Environment, Duke University, Durham, North Carolina, USA;[2]U.S. Geological Survey, Columbia Environmental Research Center, Columbia, Missouri, USA;[3]Department of Forestry, School of Natural Resources, University of Missouri, Columbia, Missouri, USA;[4]Department of Family and Community Medicine, and[5]Department of Obstetrics, Gynecology and Women's Health, School of Medicine, University of Missouri, Columbia, Missouri, USA

Background: Hydraulic fracturing technologies, developed over the last 65 years, have only recently been combined with horizontal drilling to unlock oil and gas reserves previously deemed inaccessible. Although these technologies have dramatically increased domestic oil and natural gas production, they have also raised concerns for the potential contamination of local water supplies with the approximately 1,000 chemicals that are used throughout the process, including many known or suspected endocrine-disrupting chemicals.

Objectives: We discuss the need for an endocrine component to health assessments for drilling-dense regions in the context of hormonal and antihormonal activities for chemicals used.

Methods: We discuss the literature on *a*) surface and groundwater contamination by oil and gas extraction operations, and *b*) potential human exposure, particularly in the context of the total hormonal and antihormonal activities present in surface and groundwater from natural and anthropogenic sources; we also discuss initial analytical results and critical knowledge gaps.

Discussion: In light of the potential for environmental release of oil and gas chemicals that can disrupt hormone receptor systems, we recommend methods for assessing complex hormonally active environmental mixtures.

Conclusions: We describe a need for an endocrine-centric component for overall health assessments and provide information supporting the idea that using such a component will help explain reported adverse health trends as well as help develop recommendations for environmental impact assessments and monitoring programs.

Citation: Kassotis CD, Tillitt DE, Lin CH, McElroy JA, Nagel SC. 2016. Endocrine-disrupting chemicals and oil and natural gas operations: potential environmental contamination and recommendations to assess complex environmental mixtures. Environ Health Perspect 124:256–264; http://dx.doi.org/10.1289/ehp.1409535

Introduction

A novel source of human and animal exposure to endocrine-disrupting chemicals (EDCs) is through their use in oil and gas drilling operations. EDCs are exogenous compounds that can disrupt both development and normal hormone action either directly, by interacting with hormone receptors as agonists/antagonists, or indirectly by, for example, altering endogenous hormone concentrations, delivery to receptors, modulation of endogenous hormone responses, enzyme activities, or other mechanisms (Bergman et al. 2013; Diamanti-Kandarakis et al. 2009; Zoeller et al. 2014). Importantly, oil and gas operation chemicals have been shown to act through both direct and indirect mechanisms (Andric et al. 2006; Kassotis et al. 2014; Knag et al. 2013; Thomas and Budiantara 1995). EDCs can exhibit effects at extremely low, environmentally relevant concentrations, particularly during sensitive windows when exposure can alter normal development and result in adverse health outcomes during adulthood (Vandenberg 2014; Vandenberg et al. 2012; vom Saal et al. 2007; Welshons et al. 2003). Although chemicals used in and produced by oil and gas operations include EDCs, carcinogens, radioactive compounds, and other toxicants, herein, we will focus on the unique issues posed by their endocrine-disrupting activities.

In hydraulic fracturing, millions of gallons of water, tens of thousands of gallons of chemicals, and millions of kilograms of suspended solids are injected into the ground under high pressure. Hydraulic fracturing serves to fracture the shale or coal bed layer and release trapped natural gas or oil, allowing for increased well production. Although hydraulic fracturing technologies have been developed over the last 65 years, they have only recently been combined with horizontal drilling to unlock vast new oil and gas reserves around the world that were previously deemed either inaccessible or unprofitable (Waxman et al. 2011; Wiseman 2008). Chemicals are added throughout the entire production process (including drilling, fracturing, and through closure) for a number of reasons (Table 1) (Deutch et al. 2011; Riedl et al. 2013; Waxman et al. 2011). In total, approximately 1,000 chemicals are known to be used throughout the process [U.S. Environmental Protection Agency (EPA) 2015; Waxman et al. 2011].

Following the initial injection into the well to generate fractures, a portion of the injected volume returns to the surface immediately; this fluid is known as "flow-back." The remaining fluids either permeate the shale or coal bed formation and/or return to the surface over the life of the producing well; this fluid is known as "produced water." Both types of wastewater can contain fracturing fluids, naturally occurring salts, radioactive materials, heavy metals, and other chemicals from the shale formation such as polycyclic aromatic hydrocarbons, alkenes, alkanes, and other volatile and semi-volatile organic compounds (Deutch et al. 2011; Fontenot et al. 2013; Harkness et al. 2015; Harvey et al. 1984; Maule et al. 2013; Warner et al. 2012). Wastewater is disposed of via injection wells, open evaporation pits, landfills, or treatment plants; through on-site burial; by being spread over road or fields; and/or by being treated and reused in future hydraulic fracturing operations (Deutch et al. 2011; Gilmore et al. 2014; Lee et al. 2011; Wiseman 2008). Treatment of wastewater for reuse or disposal varies by geological region owing to differing chemical compositions and may include biological treatment, filtration or aeration steps, and/or reverse-osmosis separation (Lester et al. 2015).

Address correspondence to S.C. Nagel, University of Missouri, Obstetrics, Gynecology and Women's Health, M659 Medical Sciences Building, 1 Hospital Drive, University of Missouri-Columbia, Columbia, MO 65211 USA. Telephone: (573) 884-3028. E-mail: nagels@health.missouri.edu

We wholeheartedly thank C. Kwiatkowski and J.J. Bromfield for their comments on the development of this manuscript.

The writing of this manuscript was supported by STAR Fellowship Assistance agreement no. FP-91747101 awarded by the U.S. Environmental Protection Agency (EPA) (C.D.K.).

The views expressed are those of the authors and of the U.S. Geological Survey; however, they are not necessarily the views of the U.S. EPA. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. government.

The authors declare they have no actual or potential competing financial interests.

Received: 27 November 2014; Accepted: 22 July 2015; Advance Publication: 27 August 2015; Final Publication: 1 March 2016.

2016-009474-00328

Potential Routes of Exposure to Oil and Natural Gas Operation Chemicals

Water. Oil and natural gas operations can lead to the contamination of surface and groundwater, both of which are sources of drinking water (reviewed by Brantley et al. 2014; Burton et al. 2014; Vengosh et al. 2014). There are a variety of routes of contamination: spills of chemicals during transport to and from the fracturing site, the drilling and fracturing processes, improper treatment and disposal of wastewater, failure of well casings, and structural failure in abandoned wells (Ingraffeaet al. 2014; Kell 2011; Mauter et al. 2014; Rozell and Reaven 2012).

In 2013, spills were reported at 1% of Colorado wells (550/51,000 active wells), and it has been estimated that 50% of surface spills contaminate groundwater on the basis of data from Weld County, Colorado (Gross et al. 2013). An analysis of permitted Pennsylvania wells suggests a similar total spill rate of 2% (103/5,580 active wells; Souther et al. 2014). Although all 24 states with active shale reservoirs report spills, reporting limits and required information vary widely, and only 5 states require maintenance of public records for spills and violations (Soraghan 2014; Souther et al. 2014). Given the limited mandatory reporting, it is likely that the magnitude of the impact of oil and gas operations on water quality is underestimated (Soraghan 2014; Souther et al. 2014). For example, an analysis in Pennsylvania found that industry had reported 59% of documented spills (Souther et al. 2014).

Wastewater is commonly sent to wastewater treatment plants in many regions (Gilmore et al. 2014) that are not able to remove many of the anthropogenic or naturally occurring compounds present in wastewater from shale operations (Braga et al. 2005; Campbell et al. 2006; Westerhoff et al. 2005). Following this treatment, these compounds can be discharged into surface water (Ferrar et al. 2013b; Harkness et al. 2015; Warner et al. 2013, 2014). Transportation of chemicals for drilling and fracturing to well pads and transportation of wastewater away from well pads poses risks for contamination (Burton et al. 2014). Spills and leaks occur during transportation through wastewater pipelines, transfer to trucks at well pads, and vehicular transport to disposal facilities (Gilmore et al. 2014).

Groundwater contamination associated with oil and gas operations has also been reported (Fontenot et al. 2013; Jackson et al. 2013; Osborn et al. 2011; Vengosh et al. 2014). This contamination can occur via migration of chemicals from the surface or underground. An investigation of wastewater pits and impoundments in the Marcellus Shale region reported a lack of maintenance of containment and transport systems, with spills affectinggroundwater largely as a result of equipment failures and corrosion of pipes and tanks (Ziemkiewicz et al. 2014). Surface spills of fracturing fluids can also contaminate groundwater, and elevated concentrations of benzene, toluene, ethylbenzene, and xylenes (BTEX) have been reported in groundwater near surface spills (Gross et al. 2013; Ziemkiewicz et al. 2014). A recent U.S. EPA report conclusively linked hydraulic fracturing to drinking-water contamination at wells within fiveof six retrospective study regions; no baseline testing was available for the sixth region (U.S. EPA 2015). Underground migration potential is also a concern. Concentrations of heavy metals have been shown to increase in drinking water with proximity to natural gas wells (Fontenot et al. 2013), and thermogenic (shale-origin) gas concentrations in drinking water sampled from close proximity to natural gas wells have been reported to be higher than in water sampled from more distant sources (Jackson et al. 2013; Li and Carlson 2014; Osborn et al. 2011). Recent work suggests that the main reason for these findingsmay be faulty well casings (Darrah et al. 2014).

Air. Oil and natural gas production processes also contribute contaminants to the air, creating another potential route of exposure for humans and animals (Colborn et al. 2014; Helmig et al. 2014; Macey et al. 2014; Moore et al. 2014). Potential sources of inhalation exposure for these chemicals include evaporation from surface spills and evaporation pits, flaring at the surface, and release of chemicals during surface transfers and during processing (Colborn et al. 2014; Trimble 2012). High-level releases of chemicals are episodic (Brown et al. 2014, 2015). Elevated levels of volatile organic compounds (VOCs) such as BTEX, alkenes, alkanes, aromatic compounds, and aldehydes have been reported during drilling, production, and completion from nearby wells (Colborn

Table 1. Functional categories of hydraulic fracturing chemicals [adapted from Colborn et al. (2011)].

Chemical categories	Technical hydraulic fracturing use	Example compounds
Acids	To achieve greater injection ability or penetration and later to dissolve minerals and clays to reduce clogging, allowing gas to flow to the surface.	Hydrochloric acid
Biocides	To prevent bacteria that can erode pipes and fittings and to break down gellants that serve to ensure that fluid viscosity and proppant transport are maintained.	1-methyl-4-isothiazolin-3-one, bronopol, glutaraldehyde
Breakers	To allow the breakdown of gellants used to carry the proppant; these are added near the end of the hydraulic fracturing sequence to enhance flowback.	Ammonium persulfate, magnesium peroxide
Clay stabilizers	To create a fluid barrier to prevent mobilization of clays, which can plug fractures.	Tetramethyl ammonium chloride, sodium chloride
Corrosion inhibitors	To reduce the potential for rusting in pipes and casings.	Ethoxylated octylphenol and nonylphenol, isopropanol
Crosslinkers	To thicken fluids, often with metallic salts, in order to increase viscosity and proppant transport.	Ethylene glycol, sodium tetraborate decahydrate, petroleum distillate
Defoamers	To reduce foaming after it is no longer needed in order to lower surface tension and allow trapped gas to escape.	2-ethylhexanol, oleic acid, oxalic acid
Foamers	To increase carrying capacity while transporting proppants and decreasing the overall volume of fluid needed.	2-butoxyethanol, diethylene glycol
Friction reducers	To make water slick and minimize the friction created under high pressure and to increase the rate and efficiency of moving the hydraulic fracturing fluid.	Acrylamide, ethylene glycol, petroleum distillate, methanol
Gellants	To increase viscosity and suspend sand during proppant transport.	Propylene glycol, guar gum, ethylene glycol
pH control	To maintain the pH at various stages with buffers to ensure the maximum effectiveness of various additives.	Sodium hydroxide, acetic acid
Proppants	To hold fissures open, allowing gas to flow out of the cracked formation; usually composed of sand and occasionally glass or ceramic beads.	Styrene, crystalline silica, ceramic, graphite
Scale inhibitors	To prevent buildup of mineral scale that can block fluid and gas passage through the pipes.	Acrylamide, sodium polycarboxylate
Surfactants	To decrease liquid surface tension and improve fluid passage through pipes in either direction.	Naphthalene, 1,2,4-trimethylbenzene, ethanol, methanol, 2-butoxyethanol

Categories and uses for commonly applied chemicals that are commonly used throughout the hydraulic fracturing process with specificexamples provided for each category class. Adapted with permission from Colborn T et al. (2011). Reprinted by permission of Taylor & Francis LLC (http://www.tandfonline.com).

et al. 2014; McKenzie et al. 2012; Roy et al. 2014; Steinzor et al. 2013), in some cases exceeding levels observed in heavily polluted inner cities (Helmig et al. 2014).

Endocrine-Disrupting Chemicals and Oil and Gas Operations

EDC activity of chemicals used in oil and natural gas operations. Our laboratory has tested the estrogen and androgen receptor activities of 12 chemicals commonly used in oil and gas operations using a luminescence-based reporter gene bioassay in human cancer cells. We measured stimulation of receptors (agonist) or inhibition of positive control–induced expression (antagonist). We found 1 estrogen receptor agonist, 11 estrogen receptor antagonists, and 10 androgen receptor antagonists; several chemicals exhibited multiple receptor activities (Kassotis et al. 2014).

A 2011 analysis reported approximately 120 known or suspected EDCs out of 353 oil and gas operation chemicals with Chemical Abstract Service (CAS) numbers (Colborn et al. 2011). Importantly, only half of the known oil and gas operation chemicals had CAS numbers at that time, greatly limiting the health assessment for other chemicals used in these processes (Waxman et al. 2011). Still other chemicals remain proprietary information (Shonkoff et al. 2014; Wiseman 2011). For example, a recent study found that 67%, 37%, and 18% of assessed wells were fractured with ≥ 1, 5, or 10 proprietary chemicals, respectively (Souther et al. 2014).

EDC activity in water near oil and natural gas operations. We assessed the estrogen and androgen receptor activities of water samples collected from five sites in a drilling-dense region of Garfield County, Colorado, that had experienced industry-related spills or preventable discharges relative to surface and groundwater collected immediately outside of the drilling-dense region (Kassotis et al. 2014). Analysis of these samples revealed that surface and groundwater from Garfield County spill sites contained significantly elevated estrogen agonist, estrogen antagonist, and androgen antagonist activities relative to those at reference sites (Kassotis et al. 2014). Independent analytical water testing at these sites identified chemicals that we or others have shown to exhibit these same agonist and antagonist activities (discussed by Kassotis et al. 2014). Other researchers have reported estrogen agonist and androgen antagonist activities associated with oil sands and oil production wastewater (He et al. 2011; Thomas et al. 2004, 2009; Tollefsen et al. 2007).

Concentration of oil and natural gas operation chemicals in water. Hydraulic fracturing wastewater is reported to contain hundreds of organic chemicals (polyethylene glycols, ethoxylated surfactants, BTEX compounds, biocides, polycyclic aromatic hydrocarbons, aromatic amines, and more), with total dissolved organic carbon as high as 5.5 g/L, and many individual compounds present at > 500 mg/L and up to grams per liter concentrations (Kahrilas et al. 2015; Maguire-Boyle and Barron 2014; Orem et al. 2014; Thurman et al. 2014). A recent report analyzed publicly available data on FracFocus, an industry disclosure website (http://www.fracfocus.org/), and reported benzene ≤ 4.1% and naphthalene and ethylbenzene ≤ 0.45% of total fracturing fluid volume, resulting in milligrams per liter concentrations for these and other chemicals (Schaeffer and Bernhardt 2014).

Surface spills have been reported to contaminate groundwater with chemicals from oil and gas operations (Gross et al. 2013). Groundwater at surface spill sites contained 1.4, 2.2, 0.2, and 2.6 mg/L benzene, toluene, ethylbenzene, and xylene, respectively, and these concentrations decreased over time and distance from the spill sites (Gross et al. 2013). Sampling of groundwater in Pavillion, Wyoming, by the U.S. EPA in a region where no specific accident or spill had occurred revealed concentrations of BTEX, naphthalene, ethylene glycols, and other oil and gas chemicals at concentrations ranging from 0.01 to 8 mg/L (DiGiulio et al. 2011). Because some of these chemicals have been shown to disrupt multiple hormone receptors *in vitro* at concentrations in the micrograms per liter range (Kassotis et al. 2014), these groundwater samples contained concentrations of these chemicals within the bioactive range in our reporter gene assays. To date, few comprehensive analyses have been performed of oil and gas operation–derived chemicals in drinking-water samples.

Potential Endocrine-Related Health Effects of Oil and Gas Operation Chemicals

Oil and gas operation chemicals and health effects. Evidence of potential harm from exposure to hazardous chemicals, pollutants, and emissions used in oil and natural gas operations has been reported. These reports have most often been case series involving natural experiments using quasi-experimental design and have investigated domestic animals and wildlife (Bamberger and Oswald 2012). Researchers have also begun to document in both reports and white papers the content and quantities of hazardous chemicals, pollutants, and emissions associated with these operations (Eastern Research Group and Sage Environmental Consulting 2011; Ethridge 2010; Steinzor et al. 2013; Witter et al. 2008). Concurrent with these environmental testing projects, surveys of local residents were also performed, and the reports suggested that living in close proximity to oil and gas operations has the potential to affect human and environmental health (Ferrar et al. 2013a; Rabinowitz et al. 2015; Steinzor et al. 2013; Subra 2009, 2010). At the present time, a limited number of epidemiology studies have been conducted to explore the relationship between health effects and exposure to oil and gas operation chemicals as described herein and as reviewed by Webb et al. (2014) and Werner et al. (2015).

The biological plausibility of health effects associated with exposure to hazardous chemicals, pollutants, and emissions used in oil and natural gas operations has also been explored. Many of these chemicals have documented adverse health effects in humans, are designated priority pollutants by the U.S. EPA, and/or are known or suspected EDCs (Colborn et al. 2011; Waxman et al. 2011). For example, exposure to naphthalene, a constituent of crude oil and a chemical used by industry for hydraulic fracturing processes (Waxman et al. 2011) and that has been reported in air and water near operations (Colborn et al. 2014; DiGiulio et al. 2011; Wolf Eagle Environmental 2009), can result in altered steroid hormone levels, increased reproductive abnormalities, and impaired sexual maturation in animal models and *in vitro* (Hansen et al. 2008; Pollino et al. 2009; Thomas and Budiantara 1995; Tintos et al. 2006), albeit generally at greater concentrations than those reported near these sites.

Occupational exposures. As with all environmental exposures, those who work around or with hazardous chemicals face significantly higher exposure risk than does the general population. The National Institute of Occupational Health and Safety (NIOSH) has published two studies for the oil and natural gas extraction industry: one about work crew exposures to respirable crystalline silica, and the other about work crew exposures to VOCs (Esswein et al. 2013, 2014). In both cases, these pilot data indicated that some workers' exposures exceeded NIOSH and/or ACGIH safe levels (reported therein) for crystalline silica, flammable hydrocarbon emissions, and benzene.

Reproductive effects. Exposure to VOCs including but not limited to benzene, toluene, ethylbenzene, xylenes, and formaldehyde, all chemicals used in and produced by oil and natural gas operations (Colborn et al. 2011; Waxman et al. 2011), is associated with reproductive health effects in both humans and animals. These effects include impaired fertility and fecundity via reduced semen quality and impaired menstrual cycles as well as increased risk of miscarriage, stillbirth, preterm birth, and birth defects, as reviewed by Webb et al. (2014). A list of other adverse endocrine health effects due to exposure to single chemicals used in and produced by oil and gas operations has been assembled and is available online (http://endocrinedisruption.org/chemicals-in-natural-gas-operations/chemicals).

Adverse pregnancy outcomes. McKenzie et al. (2014) used spatial analysis to evaluate the likelihood of adverse pregnancy outcomes in a cohort of 12,842 live births for mothers living within 10 miles of drilling well operations compared with mothers with no drilling wells within 10 miles. Significantly increased risks for congenital heart defects [adjusted odds ratio (AOR) = 1.3; 95% confidenceinterval (CI), 1.2, 1.5] and neural tube defects (AOR = 2.0; 95% CI: 1.0, 3.9) were observed, but no association with oral clefts (AOR = 0.82; 95% CI: 0.55, 1.2) was observed. In contrast, a study on low birth weight that used a similar design showed mixed results (McKenzie et al. 2014; Stacy et al. 2015). In two case–control studies, maternal or paternal occupational exposure to glycol ethers (hormonally active chemicals used in fracturing fluids; Kassotis et al. 2014; U.S. EPA 2015; Waxman et al. 2011) and other chemicals (pesticides, polychlorinated compounds, phthalates, bisphenol A, alkylphenolic compounds, heavy metals, and miscellaneous agents) during pregnancy was associated with congenital malformations (Cordier et al. 1997).

Cancer. In a health impact assessment, McKenzie et al. (2012) used spatial modeling based on residence proximity (≤ 0.5 miles vs. > 0.5 miles) to oil and gas operations in Colorado and found an elevated cumulative cancer risk for people living near drilling wells (10 per 1,000,000 vs. 6 per 1,000,000). Two studies calculated standardized incidence ratios. One study was a cancer cluster analysis that compared the rates for several cancers in a drilling-dense Texas town with state rates using 3 years of cancer incidence data. Mokry et al. (2010) reported a statistically significantly elevated rate for breast cancer [(standardized incidence ratio (SIR) = 1.3; 95% CI: 1.1, 1.5]. The other study compared Pennsylvania counties before and after launching drilling operations. Fryzek et al. (2013) found a slightly increased rate of one cancer, central nervous system tumors (SIR = 1.13; 95% CI: 1.02, 1.25), after unconventional drilling operations began in northeast Pennsylvania (Fryzek et al. 2013).

Limitations and data gaps. Limitations of the above-mentioned studies are the lack of both direct exposure assessment and information on residential mobility of study participants. To date, no longitudinal study has enrolled a cohort of residents in a community that has an active oil and natural gas extraction industry so that biomarkers can be obtained in a timely manner. Known and suspected risk factors need to be collected to fully model the exposure risk. The critical route/timing of exposure for hazardous chemicals associated with oil and natural gas operations has yet to be established. Drilling wells release differentamounts of air pollutants at different stages of the development and production processes (Brown et al. 2014; Colborn et al. 2014; Helmig et al. 2014; McKenzie et al. 2012), and residents, including pregnant women, may be exposed to these pollutants throughout extraction or only during specific stages. Drinking-water exposure may show considerable heterogeneity owing to the hydrogeology of undergroundwater flowasso - ciated with released natural and man-made chemicals, and limited data are available on contamination of drinking water in areas that have oil and natural gas operations.

Recommendations

Thendocrine system is designed to respond to extremely low concentrations of hormones, making it uniquely equipped to assess exposure to low levels of exogenous hormonally active contaminants. Although toxicological studies often assess adverse outcomes from high-exposure scenarios relevant to occupational exposure, endocrinological studies can assess outcomes from low-level exposure that may be more relevant to humans living near oil and natural gas operations. By combining existing *in vivo* EDC studies with knowledge of the hormone receptor activity profile of chemicals used in oil and natural gas operations, we can identify adverse health outcomes in areas where humans and animals are exposed to these chemicals for epidemiological assessment. We can then use a modified Bradford-Hill approach to assess causality between environmental exposures and adverse health outcomes, as suggested by Zoeller et al. (2014). The risks related to potential exposure and adverse outcomes in humans and wildlife populations have not been affordedcomplete evaluations in part because of exemptions from parts of six key federal regulatory acts that traditionally act to safeguard U.S. water sources, including the Safe Drinking Water Act and the Clean Water Act (Clean Water Act 1972; Deutch et al. 2011; Safe Drinking Water Act 1974).

Based on the hypothesis that exposure to oil and natural gas chemicals contributes to negative health outcomes, we offer the following recommendations to evaluate the risks posed to humans and wildlife: *a*) integrate endocrine-centric end points into human health assessments in areas of unconventional drilling operations; *b*) perform biomonitoring studies for chemicals and their metabolites in humans; *c*) develop an effect-directed screening approach to assess endocrine-related effectsof mixtures; *d*) perform controlled laboratory animal studies of exposure to complex mixtures of oil and natural gas chemicals to assess adverse health outcomes; and *e*) perform *in vitro* bioassays to assess receptor interactions with complex mixtures.

Endocrine health assessments. We suggest incorporating an endocrine-centric component into overall human and environmental health assessments. An endocrine-centric health component would assume additivity of chemicals, an assumption that has been shown to be reasonable for chemicals acting through similar mechanisms of action (Payne et al. 2000; Rajapakse et al. 2002; Silva et al. 2002). Thiapproach would assess common adverse endocrine end points that have been shown to result from disruption of specific hormone receptors alone and in combination, including *a*) reproductive effects(infertility, subfertility, reduced sperm counts, miscarriage, preterm birth, birth weight, puberty), *b*) developmental irregularities (cryptorchidism, hypospadias, neural tube defects, congenital heart defects), and *c*) cancer, particularly hormone-responsive types such as testicular, breast, prostate, and brain cancers (reviewed by Bergman et al. 2013; Diamanti-Kandarakis et al. 2009; Vandenberg et al. 2012; Zoeller et al. 2012).

Measurement of chemicals in humans and wildlife (biomonitoring). One of the major limitations in human risk assessment of oil and natural gas operations is the paucity of chemical exposure information, considering the number of chemicals used and the proprietary disclosure rules. Until now, most research has focused on airborne emissions (reviewed by Moore et al. 2014) and water contamination (reviewed by Rozell and Reaven 2012; Vengosh et al. 2014). Although epidemiological studies have begun to assess adverse health outcomes near drilling operations (McKenzie et al. 2014), to our knowledge, no researchers have yet published data on concentrations of oil and gas operation chemicals in humans or wildlife.

Chemical characterization is required to determine appropriate biomonitoring candidates. Recent work has detailed analytical approaches for characterizing the various classes of compounds present in hydraulic fracturing wastewater (Ferrer and Thurman 2015). We suggest that oil and gas wastewater be used to determine the presence of chemicals that can result in the observed agonist and/or antagonist responses. Initial identification should occur via reverse matching to known compound lists such as the National Institute of Standards and Technology (NIST) Spectral Search Program for the NIST/U.S. EPA/National Institutes of Health (NIH) Mass Spectral Library. These compounds can be further reverse-matched to known oil and gas operation chemicals (Colborn et al. 2011, 2014; U.S. EPA 2015; Waxman et al. 2011). Because this step may miss proprietary compounds not currently reported by industry, it should be used as a supplement to reverse-matching databases. These compounds can then be confirmed by comparing them with authentic standards. These chemicals can be further tested in bioassays to determine receptor activities and their likely presence and contribution to activities in water. These data can then guide the development of analytical methods for target compounds and their metabolites serving as biomonitoring candidates in humans living near extraction operations.

Using effects-directed analysis to identify chemicals responsible for EDC activity. Analytical identification of hormonally active chemicals present in both water and air must be performed to better characterize source and exposure and to assess risk. Whenever possible, analysis of complex environmental samples should be performed using an effects-directed analysis approach (Burgess et al. 2013; Liscio et al. 2014; Rostkowski et al. 2011) coupled with a response–balance approach (Cargouët et al. 2004; Schriks et al. 2010; Sun et al. 2008).

This effects-directed/response–balance approach should target the most hormonally active samples from drilling regions (as well as from reference sites to eliminate background activity/chemicals) for chemical fractionation and testing. These procedures should include orthogonal separations and screening of the resulting fractions in bioassays to refine and isolate bioactive chemicals. Refined fractions can then be analyzed using the mass spectrometry (MS) tools described below and recently reported (Ferrer and Thurman 2015) to help identify chemicals responsible for observed activities. Once candidate chemicals have been identified, authentic standards may be used to confirm the MS identification and the bioactivity observed in bioassays. This method has been used successfully to identify novel bioactive compounds and represents the best approach for characterizing the EDCs that are most responsible for observed activities (Liscio et al. 2014; Rostkowski et al. 2011). Finally, biological activity can be coupled with chemical concentrations obtained from environmental monitoring to determine relative contributions to observed receptor activities, as has been described by others (Cargouët et al. 2004; Schriks et al. 2010; Sun et al. 2008).

EDC-centric laboratory animal health assessments. Laboratory animal models can and should be used to test for causal relationships between exposure and negative health outcomes that might be expected in drilling-dense regions. Humans and wildlife living in these regions are likely exposed to oil and gas operation chemicals during different developmental windows, and known critical periods such as prenatal, perinatal, childhood, and puberty should be targeted. Studies of adult exposure should also be performed to assess occupational exposure and chronic exposure at environmentally relevant levels encountered by nearby residents. We further recommend that the route of exposure remain as relevant as possible. Likely exposure to chemicals may occur through oral, dermal, and/or inhalation routes, and parameters such as volatility and partition coefficients will help determine which exposure routes are of the highest concern for individual chemicals. Route of exposure is crucial to understanding health effects because varying routes of exposure can result in very different bioavailability of EDCs, as has recently been described for bisphenol A (Gayrard et al. 2013; Hormann et al. 2014; vom Saal and Welshons 2014). Adverse health outcomes that should be targeted are described above in both the section entitled "Potential Endocrine-Related Health Effects of Oil and Gas Operation Chemicals" as well as in our recommendation regarding endocrine health assessments and are known to result from exposure to EDCs (reviewed by Bergman et al. 2013; Diamanti-Kandarakis et al. 2009; Vandenberg et al. 2012; Zoeller et al. 2012); many protocols have been described for the evaluation of these end points (Diamanti-Kandarakis et al. 2009; Schug et al. 2013; U.S. EPA 2009a, 2009b, 2009c; Zoeller et al. 2012). These data can provide important information for further refining human epidemiological studies as well as studies on pets and wildlife populations, which have recently been shown to be affected by endocrine health concerns (Bamberger and Oswald 2012, 2014, 2015; Grant et al. 2015; Papoulias and Velasco 2013; Slizovskiy et al. 2015).

Bioassays for complex mixtures. With approximately 1,000 chemicals used in and produced by oil and gas operations (U.S. EPA 2015), there is a critical need for methods to assess the EDC activity of these complex mixtures. Methods of assessing the activity and potential health risks of mixtures that can appropriately address the interplay between receptor systems are limited. Observed outcomes *in vivo* can often be the result of disruption of several hormone receptor systems by single chemicals or by mixtures. Statistical modeling (Orton et al. 2012), *in vitro* and *in vivo* assays (Silva et al. 2002), quantitative structure analysis (Nishihara et al. 2000), gene expression (Richter et al. 2014), and other tools have been used to assess a number of laboratory-defined mixtures that interact with single hormone receptors.

Modeling complex mixtures can greatly reduce the number of independent tests that need to be performed when assessing toxicity. For example, Bertin et al. used a neural networking model to assess mixture toxicity, achieving a predictive model with approximately 10% of actual interactions tested (Bertin et al. 2013). However, despite clear successes with relatively uncomplicated mixtures, analysis of more complicated mixtures appears to be beyond current capabilities (Kortenkamp et al. 2014; Orton et al. 2012) owing to insufficient knowledge of interreceptor interactions and indirect chemical–receptor interactions (Kortenkamp et al. 2014). An additional concern involves indirect interactions between chemicals and receptors. For example, interaction with the aryl hydrocarbon receptor can result in the activation of cytochrome P450 enzymes, which are well known to alter endogenous and exogenous chemical metabolism and therefore exposure (Anzenbacher and Anzenbacherová 2001; Markowitz et al. 2003). Inactive chemicals can be metabolized into active metabolites, resulting in mixtures of inactive chemicals that can act as agonists or antagonists in mixtures only (Gauger et al. 2007). Improved characterization of these interactions will provide a clearer understanding of the utility models can provide towards assessing *in vivo* outcomes, as well as their limitations.

Because it is not possible to test all combinations of chemicals *in vitro* and/or *in vivo*, we recommend performing guided *in vitro* and *in vivo* research that focuses on receptor interactions. We suggest that reporter gene assays be used for *in vitro* testing because of their low cost, ease of use, reliability, high sensitivity, and ease of adapting for multiple receptor systems (Naylor 1999; Rajapakse et al. 2002; Silva et al. 2002; Soto et al. 2006). Similar assays including yeast receptor screens [yeast estrogen screen (YES), yeast androgen screen (YAS), etc.] tend to be less robust and less sensitive, albeit less susceptible to toxicity, whereas cell proliferation assays (E-SCREEN, A-SCREEN, etc.) are equally sensitive and, unlike reporter gene assays, can measure nongenomic effects through cell-surface receptors; however, they are generally less applicable for diverse receptor testing (Leusch et al. 2010). Current high-throughput assay options such as Tox21 or ToxCast™ are of great use as diverse first-pass screens for individual compounds, although it is unclear whether they will be helpful in the assessment of complex mixtures (Filer et al. 2014; Tice et al. 2013). Rather than the single-receptor tests used by these systems, assessing chemicals and mixtures of chemicals in controlled multiple-receptor systems is critical to understanding and accounting for receptor interplay.

Improvement of the utility of *in vitro* assay systems should take place in several steps. First, receptor interaction can be assessed through testing positive controls in both the presence and the absence of other receptors. Ideally, this testing should be done across several cell lines to identify chemical impingement on receptor interactions and tissue-specificcomodulators. Once multiple-receptor experiments are carried out with single chemicals, simple mixtures with clearly definedreceptor activity profilescan be introduced to determine how simultaneous interactions with several receptors can modulate responses. Further work should be coupled with *in vivo* experiments to understand these interactions in a whole-animal model and to confirm *in vitro* multiple-receptor results.

Potential Implications

Recent analyses of the potential contributions of EDC exposures to adverse endocrine health outcomes, such as obesity, cancers (particularly hormone-dependent), reproduction/infertility, metabolic diseases, and developmental abnormalities, suggest that EDC exposures account for an estimated 1.8% to 40% of societal health care costs (Hunt and Ferguson 2014; Olsson 2014; Trasande 2014). More recently, a suite of studies estimated the potential health care costs for the European Union (EU) due to EDC exposures: neurobehavioral deficits and disorders (> 150 billion euros; Bellanger et al. 2015), obesity and diabetes (> 18 billion euros; Legler et al. 2015), and male reproductive disorders and diseases (> 15 billion euros; Hauser et al. 2015). Altogether, the median cost to the EU for EDCs with the highest probability of causation was estimated at 157 billion euros per year (Trasande et al. 2015). Whereas exposure to oil and gas operation chemicals individually would likely result in only a fraction of these costs, increasing exposure to additional hormonally active chemicals is a cause for concern given the additive nature of many of these receptor systems. As such, there are potentially large financial implications for exposure to EDCs from their use in oil and gas operations.

Conclusions

Herein, we have provided a series of recommendations that will allow scientificallydefensible, accurate assessments of the potential endocrine-related risks from chemical exposure associated with oil and natural gas operations. We present these recommendations in light of the growing body of information regarding both chemical concentrations in the environment and adverse health outcomes reported in humans and in wildlife. We suggest that these approaches will lead to improved information for resource management decisions and will ultimately protect and improve human health.

References

Andric NL, Kostic TS, Zoric SN, Stanic BD, Andric SA, Kovacevic RZ. 2006. Effect of a PCB-based transformer oil on testicular steroidogenesis and xenobiotic-metabolizingenzymes. Reprod Toxicol 22:102–110.

Anzenbacher P, Anzenbacherová E. 2001. Cytochromes P450 and metabolism of xenobiotics. Cell Mol Life Sci 58:737–747.

Bamberger M, Oswald RE. 2012. Impacts of gas drilling on human and animal health. New Solut 22:51–77.

Bamberger M, Oswald RE. 2014. Unconventional oil and gas extraction and animal health. Environ Sci Process Impacts 16:1860–1865.

Bamberger M, Oswald RE. 2015. Long-term impacts of unconventional drilling operations on human and animal health. J Environ Sci Health A Tox Hazard Subst Environ Eng 50:447–459.

Bellanger M, Demeneix B, Grandjean P, Zoeller RT, Trasande L. 2015. Neurobehavioral deficits, diseases and associated costs of exposure to endocrine-disrupting chemicals in the European Union. J Clin Endocrinol Metab 100:1256–1266.

Bergman Å, Heindel JJ, Jobling S, Kidd KA, Zoeller RT, eds. 2013. State of the Science of Endocrine Disrupting Chemicals – 2012. World Health Organization. Available: http://apps.who.int/iris/bitstream/10665/78101/1/9789241505031_eng.pdf?ua=1 [accessed 25 February 2015].

Bertin MJ, Moeller P, Guillette LJ Jr, Chapman RW. 2013. Using machine learning tools to model complex toxic interactions with limited sampling regimes. Environ Sci Technol 47:2728–2736.

Braga O, Smythe GA, Schafer AI, Feitz AJ. 2005. Steroid estrogens in primary and tertiary wastewater treatment plants. Water Sci Technol 52:273–278.

Brantley SL, Yoxtheimer D, Arjmand S, Grieve P, Vidic R, Pollak J, et al. 2014. Water resource impacts during unconventional shale gas development: the Pennsylvania experience. Int J Coal Geol 126:140–156.

Brown DR, Lewis C, Weinberger BI. 2015. Human exposure to unconventional natural gas development: a public health demonstration of periodic high exposure to chemical mixtures in ambient air. J Environ Sci Health A Tox Hazard Subst Environ Eng 50:460–472.

Brown D, Weinberger B, Lewis C, Bonaparte H. 2014. Understanding exposure from natural gas drilling puts current air standards to the test. Rev Environ Health 29:277–292.

Burgess RM, Ho KT, Brack W, Lamoree M. 2013. Effects-directed analysis (EDA) and toxicity identification evaluation (TIE): complementary but different approaches for diagnosing causes of environmental toxicity. Environ Toxicol Chem 32:1935–1945.

Burton GA Jr, Basu N, Ellis BR, Kapo KE, Entrekin S, Nadelhoffer K. 2014. Hydraulic "fracking": are surface water impacts an ecological concern? Environ Toxicol Chem 33:1679–1689.

Campbell CG, Borglin SE, Green FB, Grayson A, Wozei E, Stringfellow WT. 2006. Biologically directed environmental monitoring, fate, and transport of estrogenic endocrine disrupting compounds in water: a review. Chemosphere 65:1265–1280.

Cargouët M, Perdiz D, Mouatassim-Souali A, Tamisier-Karolak S, Levi Y. 2004. Assessment of river contamination by estrogenic compounds in Paris area (France). Sci Total Environ 324:55–66.

Clean Water Act of 1972. 1972. Public Law 92-500.

Colborn T, Kwiatkowski C, Schultz K, Bachran M. 2011. Natural gas operations from a public health perspective. Hum Ecol Risk Assess 17:1039–1056.

Colborn T, Schultz K, Herrick L, Kwiatkowski C. 2014. An exploratory study of air quality near natural gas operations. Hum Ecol Risk Assess 20:86–105.

Cordier S, Bergeret A, Goujard J, Ha MC, Aymé S, Bianchi F, et al. 1997. Congenital malformation and maternal occupational exposure to glycol ethers. Occupational Exposure and Congenital Malformations Working Group. Epidemiology 8:355–363.

Darrah TH, Vengosh A, Jackson RB, Warner NR, Poreda RJ. 2014. Noble gases identify the mechanisms of fugitive gas contamination in drinking-water wells overlying the Marcellus and Barnett Shales. Proc Natl Acad Sci USA 111:14076–14081.

Deutch J, Holditch S, Krupp F, McGinty K, Tierney S, Yergin D, et al. 2011. The Secretary of the Energy Board Shale Gas Production Subcommittee Ninety Day Report. Washington, DC:U.S. Department of Energy. Available: http://energy.gov/sites/prod/files/Final_90_day_Report.pdf [accessed 25 February 2015].

Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, et al. 2009. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr Rev 30:293–342.

DiGiulio DC, Wilkin RT, Miller C, Oberley G. 2011. Draft Investigation of Ground Water Contamination near Pavillion, Wyoming. EPA 600/R-00/000. Ada, OK:U.S. Environmental Protection Agency, Office of Research and Development, National Risk Mangement Reserch Laboratory. Available: http://www.epa.gov/sites/production/files/documents/EPA_ReportOnPavillion_Dec-8-2011.pdf [accessed 25 February 2015].

Eastern Research Group/Sage Environmental Consulting. 2011. City of Fort Worth Natural Gas Air Quality Study. Final Report. Available: http://fortworthtexas.gov/uploadedFiles/Gas_Wells/AirQualityStudy_final.pdf [accessed 1 July 2015].

Esswein EJ, Breitenstein M, Snawder J, Kiefer M, Sieber WK. 2013. Occupational exposures to respirable crystalline silica during hydraulic fracturing. J Occup Environ Hyg 10:347–356.

Esswein EJ, Snawder J, King B, Breitenstein M, Alexander-Scott M, Kiefer M. 2014. Evaluation of some potential chemical exposure risks during flowbackoperations in unconventional oil and gas extraction: preliminary results. J Occup Environ Hyg 11:D174–D184.

Ethridge S. 2010. Texas Commission on Environmental Quality Interoffice Memorandum to Mark R. Vickery, P.G. Health Effects Review of Barnett Shale Formation Area Monitoring Projects, including Phase I (August 24–28, 2009), Phase II (October 9–16, 2009), and Phase III (November 16–20, 2009): Volatile Organic Compound (VOCs), Reduced Sulfur Compounds (RSC), Oxides of Nitrogen (NOx), and Infrared (IR) Camera Monitoring, Document No. BS0912-FR. Available: http://www.tceq.state.tx.us/assets/public/implementation/barnett_shale/2010.01.27-healthEffects-BarnettShale.pdf [accessed 4 July 2015].

Ferrar KJ, Kriesky J, Christen CL, Marshall LP, Malone SL, Sharma RK, et al. 2013a. Assessment and longitudinal analysis of health impacts and stressors perceived to result from unconventional shale gas development in the Marcellus Shale region. Int J Occup Environ Health 19:104–112.

Ferrar KJ, Michanowicz DR, Christen CL, Mulcahy N, Malone SL, Sharma RK. 2013b. Assessment of effluent contaminants from three facilities discharging Marcellus Shale wastewater to surface waters in Pennsylvania. Environ Sci Technol 47:3472–3481.

Ferrer I, Thurman EM. 2015. Chemical constituents and analytical approaches for hydraulic fracturing waters. Trends Environ Anal Chem 5:18–25.

Filer D, Patisaul HB, Schug T, Reif D, Thayer K. 2014. Test driving ToxCast: endocrine profiling for 1858 chemicals included in phase II. Curr Opin Pharmacol 19:145–152.

Fontenot BE, Hunt LR, Hildenbrand ZL, Carlton DD Jr, Oka H, Walton JL, et al. 2013. An evaluation of water quality in private drinking water wells near

2016-009474-00328

natural gas extraction sites in the Barnett Shale formation. Environ Sci Technol 47:10032–10040.
Fryzek J, Pastula S, Jiang X, Garabrant DH. 2013. Childhood cancer incidence in Pennsylvania counties in relation to living in counties with hydraulic fracturing sites. J Occup Environ Med 55:796–801.
Gauger KJ, Giera S, Sharlin DS, Bansal R, Iannacone E, Zoeller RT. 2007. Polychlorinated biphenyls 105 and 118 form thyroid hormone receptor agonists after cytochrome P4501A1 activation in rat pituitary GH3 cells. Environ Health Perspect 115:1623–1630; doi:10.1289/ehp.10328.
Gayrard V, Lacroix MZ, Collet SH, Viguié C, Bousquet-Melou A, Toutain PL, et al. 2013. High bioavailability of bisphenol A from sublingual exposure. Environ Health Perspect 121:951–956; doi:10.1289/ehp.1206339.
Gilmore KR, Hupp RL, Glathar J. 2014. Transport of hydraulic fracturing water and wastes in the Susquehanna River Basin, Pennsylvania. J Environ Eng (New York) 140: B4013002; doi:10.1061/(ASCE)EE.1943-7870.0000810.
Grant CJ, Weimer AB, Marks NK, Perow ES, Oster JM, Brubaker KM, et al. 2015. Marcellus and mercury: assessing potential impacts of unconventional natural gas extraction on aquatic ecosystems in northwestern Pennsylvania. J Environ Sci Health A Tox Hazard Subst Environ Eng 50:482–500.
Gross SA, Avens HJ, Banducci AM, Sahmel J, Panko JM, Tvermoes BE. 2013. Analysis of BTEX groundwater concentrations from surface spills associated with hydraulic fracturing operations. J Air Waste Manag Assoc 63:424–432.
Hansen BH, Altin D, Vang SH, Nordtug T, Olsen AJ. 2008. Effects of naphthalene on gene transcription in *Calanus finmarchicus*(Crustacea: Copepoda). Aquat Toxicol 86:157–165.
Harkness JS, Dwyer GS, Warner NR, Parker KM, Mitch WA, Vengosh A. 2015. Iodide, bromide, and ammonium in hydraulic fracturing and oil and gas wastewaters: environmental implications. Environ Sci Technol 49:1955–1963.
Harvey TG, Matheson TW, Pratt KC. 1984. Chemical class separation of organics in shale oil by thin-layer chromatography. Anal Chem 56:1277–1281.
Hauser R, Skakkebaek NE, Hass U, Toppari J, Juul A, Andersson AM, et al. 2015. Male reproductive disorders, diseases, and costs of exposure to endocrine-disrupting chemicals in the European Union. J Clin Endocrinol Metab 100:1267–1277.
He Y, Wiseman SB, Hecker M, Zhang X, Wang N, Perez LA, et al. 2011. Effect of ozonation on the estrogenicity and androgenicity of oil sands process-affected water. Environ Sci Technol 45:6268–6274.
Helmig D, Thompson CR, Evans J, Boylan P, Hueber J, Park JH. 2014. Highly elevated atmospheric levels of volatile organic compounds in the Uintah Basin, Utah. Environ Sci Technol 48:4707–4715.
Hormann AM, vom Saal FS, Nagel SC, Stahlhut RW, Moyer CL, Ellersieck MR, et al. 2014. Holding thermal receipt paper and eating food after using hand sanitizer results in high serum bioative and urine total levels of bisphenol A (BPA). PloS One 9:e110509; doi:10.1371/journal.pone.0110509.
Hunt A, Ferguson J. 2014. Health Costs in the European Union: How Much is Related to EDCs? Health and Environment Alliance (HEAL). Available: http://env-health.org/IMG/pdf/18062014_final_health_costs_in_the_european_union_how_much_is_realted_to_edcs-2.pdf [accessed 25 February 2015].
Ingraffea AR, Wells MT, Santoro RL, Shonkoff SB. 2014. Assessment and risk analysis of casing and cement impairment in oil and gas wells in Pennsylvania, 2000–2012. Proc Natl Acad Sci USA 111:10955–10960.
Jackson RB, Vengosh A, Darrah TH, Warner NR, Down A, Poreda RJ, et al. 2013. Increased stray gas abundance in a subset of drinking water wells near Marcellus shale gas extraction. Proc Natl Acad Sci USA 110:11250–11255.
Kahrilas GA, Blotevogel J, Stewart PS, Borch T. 2015. Biocides in hydraulic fracturing fluids: a critical review of their usage, mobility, degradation, and toxicity. Environ Sci Technol 49:16–32.
Kassotis CD, Tillitt DE, Davis JW, Hormann AM, Nagel SC. 2014. Estrogen and androgen receptor activities of hydraulic fracturing chemicals and surface and ground water in a drilling-dense region. Endocrinology 155:897–907.
Kell S. 2011. State Oil and Gas Agency Groundwater Investigations and their Role in Advancing Regulatory Reforms. A Two-State Review: Ohio and Texas. Groundwater Protection Council. Available: https://fracfocus.org/sites/default/files/publications/state_oil__gas_agency_groundwater_investigations_optimized.pdf [accessed 25 February 2015].
Knag AC, Verhaegen S, Ropstad E, Mayer I, Meier S. 2013. Effects of polar oil related hydrocarbons on steroidogenesis *in vitro* in H295R cells. Chemosphere 92:106–115.
Kortenkamp A, Scholze M, Ermler S. 2014. Mind the gap: can we explain declining male reproductive health with known antiandrogens? Reproduction 147:515–527.
Lee DS, Herman JD, Elsworth D, Kim HT, Lee HS. 2011. A critical evaluation of unconventional gas recovery from the Marcellus shale, northeastern United States. KSCE J Civil Eng15:679–687.
Legler J, Fletcher T, Govarts E, Porta M, Blumberg B, Heindel JJ, et al. 2015. Obesity, diabetes, and associated costs of exposure to endocrine-disrupting chemicals in the European Union. J Clin Endocrinol Metab 100:1278–1288.
Lester Y, Ferrer I, Thurman EM, Sitterley KA, Korak JA, Aiken G, et al. 2015. Characterization of hydraulic fracturing flowback water in Colorado: implications for water treatment. Sci Total Environ 512-513:637–644.
Leusch FD, de Jager C, Levi Y, Lim R, Puijker L, Sacher F, et al. 2010. Comparison of five in vitro bioassays to measure estrogenic activity in environmental waters. Environ Sci Technol 44:3853–3860.
Li H, Carlson KH. 2014. Distribution and origin of groundwater methane in the Wattenberg oil and gas fieldof northern Colorado. Environ Sci Technol 48:1484–1491.
Liscio C, Abdul-Sada A, Al-Salhi R, Ramsey MH, Hill EM. 2014. Methodology for profiling anti-androgen mixtures in river water using multiple passive samplers and bioassay-directed analyses. Water Res 57:258–269.
Macey GP, Breech R, Chernaik M, Cox C, Larson D, Thomas D, et al. 2014. Air concentrations of volatile compounds near oil and gas production: a community-based exploratory study. Environ Health 13:82; doi:10.1186/1476-069X-13-82.
Maguire-Boyle SJ, Barron AR. 2014. Organic compounds in produced waters from shale gas wells. Environ Sci Process Impacts 16:2237–2248.
Markowitz JS, Donovan JL, DeVane CL, Taylor RM, Ruan Y, Wang JS, et al. 2003. Effect of St John's wort on drug metabolism by induction of cytochrome P450 3A4 enzyme. JAMA 290:1500–1504.
Maule AL, Makey CM, Benson EB, Burrows IJ, Scammell MK. 2013. Disclosure of hydraulic fracturing fluidchemical additives: analysis of regulations. New Solut 23:167–187.
Mauter MS, Alvarez PJ, Burton A, Cafaro DC, Chen W, Gregory KB, et al. 2014. Regional variation in water-related impacts of shale gas development and implications for emerging international plays. Environ Sci Technol 45:8298–8306.
McKenzie LM, Guo R, Witter RZ, Savitz DA, Newman LS, Adgate JL. 2014. Birth outcomes and maternal residential proximity to natural gas development in rural Colorado. Environ Health Perspect 122:412–417; doi:10.1289/ehp.1306722.
McKenzie LM, Witter RZ, Newman LS, Adgate JL. 2012. Human health risk assessment of air emissions from development of unconventional natural gas resources. Sci Total Environ 424:79–87.
Mokry BJ. 2010. Summary of Investigation into the Occurrence of Cancer, Zip Codes 75022 and 75028, Flower Mound, Denton County, Texas, 1998–2007, 2007–2009. Department of State Health Services Texas Cancer Registry. Available: https://www.dshs.state.tx.us/epitox/consults/flower_mound32010.doc [accessed 27 February 2015].
Moore CW, Zielinska B, Pétron G, Jackson RB. 2014. Air impacts of increased natural gas acquisition, processing, and use: a critical review. Environ Sci Technol 48:8349–8359.
Naylor LH. 1999. Reporter gene technology: the future looks bright. Biochem Pharmacol 58:749–757.
Nishihara T, Nishikawa JI, Kanayama T, Dakeyama F, Saito K, Imagawa M, et al. 2000. Estrogenic activities of 517 chemicals by yeast two-hybrid assay. J Health Sci 46:282–298.
Olsson IM. 2014. The Cost of Inaction—A Socioeconomic Analysis of Costs Linked to Effects of Endocrine Disrupting Substances on Male Reproductive Health. Copenhagen, Denmark:Nordic Council of Ministers. Available: http://norden.diva-portal.org/smash/get/diva2:763442/FULLTEXT04.pdf [accessed 21 February 2015].
Orem W, Tatu C, Varonka M, Lerch H, Bates A, Engle M, et al. 2014. Organic substances in produced and formation water from unconventional natural gas extraction in coal and shale. Int J Coal Geol 126:20–31.
Orton F, Rosivatz E, Scholze M, Kortenkamp A. 2012. Competitive androgen receptor antagonism as a factor determining the predictability of cumulative antiandrogenic effects of widely used pesticides. Environ Health Perspect 120:1578–1584; doi:10.1289/ehp.1205391.
Osborn SG, Vengosh A, Warner NR, Jackson RB. 2011. Methane contamination of drinking water accompanying gas-well drilling and hydraulic fracturing. Proc Natl Acad Sci USA 108:8172–8176; doi:10.1073/pnas.1100682108.
Papoulias DM, Velasco AL. 2013. Histopathological analysis of fishfrom Acorn Fork Creek, Kentucky, exposed to hydraulic fracturing fluid releases. Southeast Nat 12:92–111.
Payne J, Rajapakse N, Wilkins M, Kortenkamp A. 2000. Prediction and assessment of the effects of mixtures of four xenoestrogens. Environ Health Perspect 108:983–987.
Pollino CA, Georgiades E, Holdway DA. 2009. Physiological changes in reproductively active rainbowfish (*Melanotaenia fluviatilis*) following exposure to naphthalene. Ecotoxicol Environ Saf 72:1265–1270.
Rabinowitz PM, Slizovskiy IB, Lamers V, Trufan SJ, Holford TR, Dziura JD, et al. 2015. Proximity to natural gas wells and reported health status: results of a household survey in Washington County, Pennsylvania. Environ Health Perspect 123:21–26; doi:10.1289/ehp.1307732.
Rajapakse N, Silva E, Kortenkamp A. 2002. Combining xenoestrogens at levels below individual no-observed-effect concentrations dramatically enhances steroid hormone action. Environ Health Perspect 110:917–921.

Richter CA, Martyniuk CJ, Annis ML, Brumbaugh WG, Chasar LC, Denslow ND, et al. 2014. Methylmercury-induced changes in gene transcription associated with neuroendocrine disruption in largemouth bass (*Micropterus salmoides*). Gen Comp Endocrinol 203:215–224.
Riedl J, Rotter S, Faetsch S, Schmitt-Jansen M, Altenburger R. 2013. Proposal for applying a component-based mixture approach for ecotoxicological assessment of fracturing fluids.Environ Earth Sci 70:3907–3920.
Rostkowski P, Horwood J, Shears JA, Lange A, Oladapo FO, Besselink HT, et al. 2011. Bioassay-directed identificationof novel antiandrogenic compounds in bile of fish exposed to wastewater effluents. Environ Sci Technol 45:10660–10667.
Roy AA, Adams PJ, Robinson AL. 2014. Air pollutant emissions from the development, production, and processing of Marcellus Shale natural gas. J Air Waste Manag Assoc 64:19–37.
Rozell DJ, Reaven SJ. 2012. Water pollution risk associated with natural gas extraction from the Marcellus Shale. Risk Anal 32:1382–1393.
Safe Drinking Water Act of 1974. 1974. Public Law 93–523.
Schaeffer E, Bernhardt C. 2014. Fracking's Toxic Loophole. Thanks to the "Halliburton Loophole," Hydraulic Fracturing Companies Are Injecting Chemicals More Toxic than Diesel. Environmental Integrity Project. Available: http://environmentalintegrity.org/wp-content/uploads/FRACKINGS-TOXIC-LOOPHOLE.pdf [accessed 25 February 2015].
Schriks M, van Leerdam JA, van der Linden SC, van der Burg B, van Wezel AP, de Voogt P. 2010. High-resolution mass spectrometric identification and quantificationof glucocorticoid compounds in various wastewaters in the Netherlands. Environ Sci Technol 44:4766–4774.
Schug TT, Abagyan R, Blumberg B, Collins TJ, Crews D, DeFur PL, et al. 2013. Designing endocrine disruption out of the next generation of chemicals. Green Chem 15:181–198.
Shonkoff SB, Hays J, Finkel ML. 2014. Environmental public health dimensions of shale and tight gas development. Environ Health Perspect 122:787–795; doi:10.1289/ehp.1307866.
Silva E, Rajapakse N, Kortenkamp A. 2002. Something from "nothing"—eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. Environ Sci Technol 36:1751–1756.
Slizovskiy IB, Conti LA, Trufan SJ, Reif JS, Lamers VT, Stowe MH, et al. 2015. Reported health conditions in animals residing near natural gas wells in southwestern Pennsylvania. J Environ Sci Health A Tox Hazard Subst Environ Eng 50:473–481.
Soraghan M. 2014. Oil and Gas: Spills up 17 Percent in U.S. in 2013. EnergyWire (Washington, DC) 12 May. Available: http://www.eenews.net/stories/1059999364 [accessed 25 February 2015].
Soto AM, MaffiniMV, Schaeberle CM, Sonnenschein C. 2006. Strengths and weaknesses of *in vitro* assays for estrogenic and androgenic activity. Best Pract Res Clin Endocrinol Metab 20:15–33.
Souther S, Tingley MW, Popescu VD, Hayman DT, Ryan ME, Graves TA, et al. 2014. Biotic impacts of energy development from shale: research priorities and knowledge gaps. Front Ecol Environ 12:330–338.
Stacy SL, Brink LL, Larkin JC, Sadovsky Y, Goldstein BD, Pitt BR, et al. 2015. Perinatal outcomes and unconventional natural gas operations in Southwest Pennsylvania. PloS One 10:e0126425; doi:10.1371/journal.pone.0126425.
Steinzor N, Subra W, Sumi L. 2013. Investigating links between shale gas development and health impacts through a community survey project in Pennsylvania. New Solut 23:55–83.
Subra W. 2009. Health Survey Results of Current and Former Dish/Clark, Texas Residents. Washington, DC:Earthworks' Oil and Gas Accountability Project. Available: https://www.earthworksaction.org/files/publications/DishTXHealthSurvey_FINAL_hi.pdf [accessed 22 July 2015].
Subra W. 2010. Community Health Survey Results: Pavillion, Wyoming. Washington, DC:Earthworks' Oil and Gas Accountability Project. Available: https://www.earthworksaction.org/files/publications/PavillionFINALhealthSurvey-201008.pdf [accessed 22 July 2015].
Sun Q, Deng S, Huang J, Shen G, Yu G. 2008. Contributors to estrogenic activity in wastewater from a large wastewater treatment plant in Beijing, China. Environ Toxicol Pharmacol 25:20–26.
Thomas KV, Balaam J, Hurst MR, Thain JE. 2004. Identification of in vitro estrogen and androgen receptor agonists in North Sea offshore produced water discharges. Environ Toxicol Chem 23:1156–1163.
Thomas KV, Langford K, Petersen K, Smith AJ, Tollefsen KE. 2009. Effect-directed identification of naphthenic acids as important in vitro xeno-estrogens and anti-androgens in North Sea offshore produced water discharges. Environ Sci Technol 43:8066–8071.
Thomas P, Budiantara L. 1995. Reproductive life history stages sensitive to oil and naphthalene in Atlantic croaker. Mar Environ Res 39:147–150.
Thurman EM, Ferrer I, Blotevogel J, Borch T. 2014. Analysis of hydraulic fracturing flowback and produced waters using accurate mass: identification of ethoxylated surfactants. Anal Chem 86:9653–9661.
Tice RR, Austin CP, Kavlock RJ, Bucher JR. 2013. Improving the human hazard characterization of chemicals: a Tox21 update. Environ Health Perspect 121:756–765; doi:10.1289/ehp.1205784.
Tintos A, Gesto M, Alvarez R, Miguez JM, Soengas JL. 2006. Interactive effects of naphthalene treatment and the onset of vitellogenesis on energy metabolism in liver and gonad, and plasma steroid hormones of rainbow trout *Oncorhynchus mykiss*. Comp Biochem Physiol C Toxicol Pharmacol 144:155–165.
Tollefsen KE, Harman C, Smith A, Thomas KV. 2007. Estrogen receptor (ER) agonists and androgen receptor (AR) antagonists in effluents from Norwegian North Sea oil production platforms. Mar Pollut Bull 54:277–283.
Trasande L. 2014. Further limiting bisphenol A in food uses could provide health and economic benefits. Health Aff (Millwood) 33:316–323.
Trasande L, Zoeller RT, Hass U, Kortenkamp A, Grandjean P, Myers JP, et al. 2015. Estimating burden and disease costs of exposure to endocrine-disrupting chemicals in the European Union. J Clin Endocrinol Metab 100:1245–1255.
Trimble DC. 2012. Unconventional Oil and Gas Development: Key Environmental and Public Health Requirements. U.S. Government Accountability Office. GAO-12-874. Available: http://www.gao.gov/products/GAO-12-874 [accessed 25 February 2015].
U.S. EPA (U.S. Environmental Protection Agency). 2009a. Endocrine Disruptor Screening Program Test Guidelines – OPPTS 890.1100: Amphibian Metamorphosis (Frog). EPA 740-C-09-002. Available: http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0576-0002 [accessed 15 July 2015].
U.S. EPA. 2009b. Endocrine Disruptor Screening Program Test Guidelines – OPPTS 890.1350: Fish Short-Term Reproduction Assay. EPA 740-C-09-007. Available: http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0576-0007 [accessed 15 July 2015].
U.S. EPA. 2009c. Endocrine Disruptor Screening Program Test Guidelines – OPPTS 890.1500: Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats. EPA 740-C-09-012. Available: http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0576-0010 [accessed 15 July 2015].
U.S. EPA. 2015. Assessment of the Potential Impacts of Hydraulic Fracturing for Oil and Gas on Drinking Water Resources (External Review Draft). EPA/600/R-15/047. Washington, DC:U.S. EPA. Available: http://cfpub.epa.gov/ncea/hfstudy/recordisplay.cfm?deid=244651 [accessed 1 August 2015].
Vandenberg LN. 2014. Non-monotonic dose responses in studies of endocrine disrupting chemicals: bisphenol A as a case study. Dose Response 12:259–276.
Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr, Lee DH, et al. 2012. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr Rev 33:378–455.
Vengosh A, Jackson RB, Warner N, Darrah TH, Kondash A. 2014. A critical review of the risks to water resources from unconventional shale gas development and hydraulic fracturing in the United States. Environ Sci Technol 48:8334–8348.
vom Saal FS, Akingbemi BT, Belcher SM, Birnbaum LS, Crain DA, Eriksen M, et al. 2007. Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. Reprod Toxicol 24:131–138.
vom Saal FS, Welshons WV. 2014. Evidence that bisphenol A (BPA) can be accurately measured without contamination in human serum and urine, and that BPA causes numerous hazards from multiple routes of exposure. Mol Cell Endocrinol 398:101–113.
Warner NR, Christie CA, Jackson RB, Vengosh A. 2013. Impacts of shale gas wastewater disposal on water quality in western Pennsylvania. Environ Sci Technol 47:11849–11857.
Warner NR, Darrah TH, Jackson RB, Millot R, Kloppmann W, Vengosh A. 2014. New tracers identify hydraulic fracturing fluidsand accidental releases from oil and gas operations. Environ Sci Technol 48:12552–12560.
Warner NR, Jackson RB, Darrah TH, Osborn SG, Down A, Zhao K, et al. 2012. Geochemical evidence for possible natural migration of Marcellus Formation brine to shallow aquifers in Pennsylvania. Proc Natl Acad Sci USA 109:11961–11966.
Waxman HA, Markey EJ, DeGette D. 2011. Chemicals Used in Hydraulic Fracturing. U.S. House of Representatives. Committee on Energy and Commerce. Minority Staff Report. Available: http://www.conservation.ca.gov/dog/general_information/Documents/Hydraulic%20Fracturing%20Report%204%2018%2011.pdf [accessed 25 February 2015].
Webb E, Bushkin-Bedient S, Cheng A, Kassotis CD, Balise V, Nagel SC. 2014. Developmental and reproductive effects of chemicals associated with unconventional oil and natural gas operations. Rev Environ Health 29:307–318.
Welshons WV, Thayer KA, Judy BM, Taylor JA,

Curran EM, vom Saal FS. 2003. Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. Environ Health Perspect 111:994–1006; doi:10.1289/ehp.5494.

Werner AK, Vink S, Watt K, Jagals P. 2015. Environmental health impacts of unconventional natural gas development: a review of the current strength of evidence. Sci Total Environ 505:1127–1141.

Westerhoff P, Yoon Y, Snyder S, Wert E. 2005. Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes. Environ Sci Technol 39:6649–6663.

Wiseman HJ. 2008. Untested waters: the rise of hydraulic fracturing in oil and gas production and the need to revisit regulation. Fordham Environ Law Rev 20:115–169.

Wiseman HJ. 2011. Trade secrets, disclosure, and dissent in a fracturing energy revolution. Columbia Law Rev 111:1–13.

Witter R, Stinson K, Sackett H, Putter S, Kinney G, Teitelbaum D, et al. 2008. Potential Exposure-Related Human Health Effects of Oil and Gas Development: A Literature Review (2003–2008). University of Colorado School of Public Health. Available: PDF available: http://docs.nrdc.org/health/files/hea_08091702b.pdf [accessed 15 July 2015].

Wolf Eagle Environmental. 2009. Town of Dish, Texas Ambient Air Monitoring Analysis. Final Report. Available: http://townofdish.com/objects/DISH_-_final_report_revised.pdf [accessed 22 July 2015].

Ziemkiewicz PF, Quaranta JD, Darnell A, Wise R. 2014. Exposure pathways related to shale gas development and procedures for reducing environmental and public risk. J Nat Gas Sci Eng 16:77–84.

Zoeller RT, Bergman A, Becher G, Bjerregaard P, Bornman R, Brandt I, et al. 2014. A path forward in the debate over health impacts of endocrine disrupting chemicals. Environ Health 14:118; doi:10.1186/1476-069X-13-118.

Zoeller RT, Brown TR, Doan LL, Gore AC, Skakkebaek NE, Soto AM, et al. 2012. Endocrine-disrupting chemicals and public health protection: a statement of principles from The Endocrine Society. Endocrinology 153:4097–4110.